

IN THE SPECIFICATION

Please amend the paragraph beginning at page 11, line 16, as follows:

The polynucleotide of the present invention may present as RNA or DNA, the latter includes cDNA, genomic DNA, and synthesized DNA. DNA can be double chains or the single chain (sense strand and/or antisense strand). The polynucleotide sequence encoding a polypeptide may be the same as in SEQ ID No.1, or a different sequence due to the redundant genetic code, which encodes the mature polypeptide in ~~SEQ ID No.2~~ SEQ ID No.3.

Please amend the paragraph beginning at page 11, line 22, as follows:

The present invention further relates to a variant of the polynucleotide encoding the amino acid sequence, polypeptide fragment, and analogue of the ~~SEQ ID No.2~~ SEQ ID No.3. These include polynucleotide variants, natural allele variants, or unnatural polynucleotide variants.

Please amend the paragraph beginning at page 12, line 9, as follows:

The present invention concerns the polypeptide encoding the amino acid sequence of the ~~SEQ ID No.2~~ SEQ ID No. 3, the activate fragment thereof, analogue, and variant.

Please amend the paragraph beginning at page 12, line 12, as follows:

The present invention relates to the polypeptide amino acid sequence of ~~SEQ ID No.2~~ SEQ ID No.3 and the active fragment, analogue and deivant. The fragment with

the same biological activity of ~~SEQ ID No. 2~~ SEQ ID No. 3 polypeptide is a fragment with essentially preserved biological function or activity.

Please amend the paragraph beginning at page 12, line 17, as follows:

The CCII prepared in this invention is a recombinant protein, polypeptide or fragment, derivative, and analogue. In particular, the polypeptide fragment, ~~variantvariant~~ variant, or analogue of ~~SEQ ID No. 2~~ SEQ ID No. 3, might be: (i) a polypeptide with one or several amino acid residues replaced by conservative or non-conservative amino acid residues (a conservative amino acid residue is preferable), where the replaced amino acid residue may or may not be encoded by the genetic code. For example, the mutant or the equivalent of CCII may be obtained by inserting, replacing and/or deleting the amino acid residue. The conservative replacement is based on the similarity in terms of the equivalent charge, solubility, hydrophobicity, and/or amphipathy of the amino acid residues, as long as it maintains the activity of CCII; or (ii) a polypeptide with one or several amino acid residues containing a substituted group; or (iii) a mature polypeptide or other functional compound, such as the polypeptide fused by compounds (for example polyethylene glycol) capable of increasing the half life of the polypeptide; or (iv) a mature polypeptide and polypeptide fusing with other amino acid sequences which render the amino acid and protein sequence helpful in purifying the mature protein. The structures helpful to purification include NTA mealty affinity chramography, such as the histidine-tryptophan module for purification on the immobilized metal; protein A structure field for purification of the immobilized immunoglobulin, and structure fields for the FLAGS extension/affiliation purification system (IMMUNEX company, Seattle, Wash). The junction sequence specific to XA

enterokinase is also helpful in the protein purification (Porath, J. et al. (1992), Prot.Exp.Purif. 3:263-281).

Please amend the paragraph beginning at page 13, line 10, as follows:

The CCII in the text of the ~~presnet~~ present invention comprises the CCII of ~~SEQ ID No.2~~ SEQ ID No.3, i.e., mature polypeptide, the polypeptide having at least 90% similarity to the polypeptide of SEQ ID No.2, 90% identity is preferable), and more preferable, having at least 95% similarity, and 95% identity is preferable. It also includes part of said polypeptide containing at least 30 amino acids, preferably having more than at least 50 amino acids.

Please amend the paragraph beginning at page 26, line 1, as follows:

col2a1-1F 5'- TCT ATC GCG CAC CCG TTG TGC -3'; SEQ ID No.4

col2a1-1R 5'- GTC TTG TAG TGC TAC GGC TTG C -3'; SEQ ID No.5

col2a-12F 5'- TTG CAG ATG TCT CCA ATA CCA G -3'; SEQ ID No.6

col2a1-2R 5'- GCA CAA CGG CTC GGG CAA TGT GCT AAC G -3'; SEQ ID No.7

col2a1-3F 5'- GCT CGG AAG CAA CGG CCT CG -3'; SEQ ID No.8

col2a1-3R 5'- CTC GTC CCG GAC GCG ACG G -3'; SEQ ID No. 9

col2a1-4F 5'- CGC TGC GAT CGT CAT GCG G -3'; SEQ ID No.10

col2a1-4R 5'- GTA GTG ACC CTA CGC CCG AG -3'; SEQ ID No.11

col2a1-5F 5'- ACG CCG GCT CTC GTG CTC CTC GTG GTG C -3'; SEQ ID No.12

col2a1-5R 5'- CCG CCC GGG TCC GAA TGC CCG CAT -3'; SEQ ID No.

Please amend the paragraph beginning at page 27, line 1, as follows:

PgF and PgR were used as sense and antisense primers for CCOL2A1 C-terminal cloning. The sequences of primers were as follows: PgF 5' CCA GGC AAG GAT GGC GCA CG 3' (SEQ ID No.14) ; PgR 5'CCT GAT CGG CTC CGC CAA TGT CCA TAG G 3' (SEQ ID No.15). PCR of CCOL2A1 genomic DNA was performed using LA Taq polymerase in GC buffer. PCR products were examined by 0.8% agarose gel electrophoresis, target gene fragments were recovered from gel slices using a gel extraction kit, and inserted in pGEM-T ~~vectors~~ vectors. The inserts were identified by restriction enzyme digestion and sequencing. Due to the high GC content in the CCOL2A1 genomic DNA and polyT structure, fragment cloning of N-terminal of CCOL2A1 genomic sequences were performed by the SOE-PCR method using the total DNA extracted from whole blood and spliced to generate a nearly full-length CCOL2A1 genomic DNA sequence.

Please amend the paragraph beginning at page 29, line 32, as follows:

CCOL2A1 3' UTR was confirmed to specially belong to CCOL2A1 via Blast database searching. Therefore, the CCOL2A1 cDNA 3'UTR was used as an amplifying subject to investigate  $\alpha 1$  (II) expression in the developing chicken embryo. Heart, liver, vitreum, cornea, skin, pectoralis, sternum and etc. in 17-day old chicken embryo were analyzed using RT-PCR. The primers were col2a1-1F and col2a1-1R, listed in Table 1. The PCR products were inserted into a pGEMT-easy vector and sequenced after restriction enzyme digestion. Simultaneously, GAPDH was amplified as an internal control using the primers PF<sub>GAPDH</sub> 5' GC AGA GGT GCT GCC CAG AAC 3'(SEQ ID No.16); PR<sub>GAPDH</sub> 5' TCA CTC CTT GGA TGC CAT GTG 3' (SEQ ID No.17) to generate a 412bp GAPDH fragment.

Please amend the paragraph beginning at page 33, line 12, as follows:

1) The construction of the eukaryotic expression vector pPICZαB/CCOL2A1

The eukaryotic expression vector was constructed as follows: PCR was performed using the primers (sequence 25 5'GGT ACC TTG GTG GAA ACT TTG CGG 3'; SEQ ID No.18) and (sequence 26 5'GGT ACC GTT ACA AGA AGC AGA CTG 3'; SEQ ID No.19), and pGEM-T/CCOL2A1 as template. The PCR mixture contained 25 µl of 2×GC Buffer I, 4 µl of dNTP (2.5 mM), 0.5 µl of 20 µM primers.

Please amend the paragraph beginning at page 37, line 21, as follows:

According to the chicken P4Hα gene sequence, primer a: 5'AGA TAC TGC TAC GAA AGA CCC CGA G 3' (SEQ ID No.20) and b: 5' CTC TCT TGG TTG TAG CCC TCA TCT G 3' (SEQ ID No.21) were designed. The PCR system contained 5 µl of 10×PCR Buffer, 5 µl of 25 mM MgCl<sub>2</sub>, 4 µl of 2.5 mM dNTP Mix, 0.5 µl of 20 µM primers, 1 µl of cDNA, 0.5 µl Taq, and water to a final volume of 50 µl. PCR products were examined by 1% agarose electrophoresis.

Please amend the paragraph beginning at page 38, line 6, as follows:

The P4Hα PCR product described above was ligated with pGEM-T, resulting in a pGEM-T/P4Hα plasmid. A *Not* I restriction site was introduced by PCR. The primers a: 5'GCG GCC GCA GAT ACT GCT ACG AAA G 3' (SEQ ID No.22) and b: 5'GCG GCC GCC TCT CTT GGT TGT AGG 3' (SEQ ID No.23), and Pfu DNA polymerase were used for PCR. PCR products were given polyA tails then ligated with a pGEM-T vector and sequenced. The target fragments were recovered after the pGEM-T/P4Hα was digested by *Not* I. The P4Hα gene fragment was ligated with the pPIC9K vector

linearized by *Not* I using T4 DNA ~~ligase~~ ligase resulting in a pPIC9K/P4H $\alpha$  expression vector. The direction of insert was identified.

Please amend the paragraph beginning at page 38, line 15, as follows:

3) P4H $\beta$  cDNA cloning and recombinant expression vector construction

According to the known chicken P4H $\beta$  subunit gene sequence, the primers F: 5'GCG GCC GCA CAG CCC CTG GAG GAG 3' (SEQ ID No.24) and R: 5'GCG GCC GCG GTG ATG TAG ATC AGT C 3' (SEQ ID No.25) were designed and *Not* I restriction sites were introduced. RNA was extracted from sternum of 17-day-old chicken embryos followed by RT-PCR. 1.5 mM [Mg<sup>2+</sup>] was used in the PCR system.

Please amend the paragraph beginning at page 39, line 20, as follows:

PCR was performed using the plasmids pGEM-T/P4H $\alpha$  and pGEM-T/P4H $\beta$  in Example 8 and 9, as templates. The primers' sequences were P4H $\alpha$ : F: 5'GCGGCCGC GAT ACT GCT ACG AAA G3' (SEQ ID No.26); R: 5'GCGGCCGC CTC CAA CTC TGA TAA C 3' (SEQ ID No.27); P4H $\beta$ : F: 5'GCGGCCGC CAG CCC CTG GAG GAG -3 (SEQ ID No.28); and R: 5' GCGGCCGC TTA ATC ATC ATC AGC 3' (SEQ ID No.29).

Please amend the paragraph beginning at page 40, line 14, as follows:

Since the P4H $\beta$  expression unit was located upstream, PCR was used to examine whether the direction of P4H $\alpha$  and  $\beta$  was coincident. The upstream primer (5' GCGGCCGC CAG CCC CTG GAG GAG 3') (SEQ ID No.28) and downstream primer (5' GCGGCCGC CTC CAA CTC TGA TAA C 3') (SEQ ID No.27) for amplifying

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P4H $\alpha$  was used to PCR. If the directions of inserts were correct, 4644bp PCR products would be apparent, otherwise, there would be no expression band.

Please delete the original Sequence Listing.

Page 45 (Abstract), after the last line, beginning on a new page, please insert the attached substitute Sequence Listing.